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Clinical Research Article

WFS1 Gene–associated Diabetes Phenotypes and Identification of a Founder Mutation in Southern India

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Abstract

Context: Wolfram syndrome (WFS) is a rare autosomal recessive disorder characterized by juvenile-onset diabetes, diabetes insipidus, optic atrophy, deafness, and progressive neurodegeneration. However, due to the progressive nature of the disease and a lack of complete clinical manifestations, a confirmed diagnosis of WFS at the time of onset of diabetes is a challenge.

Objective: With *WFS1* rare heterozygous variants reported in diabetes, there is a need for comprehensive genetic screening strategies for the early diagnosis of WFS and delineating the phenotypic spectrum associated with the *WFS1* gene variants in young-onset diabetes.

Methods: This case series of 11 patients who were positive for *WFS1* variants were identified with next-generation sequencing (NGS)–based screening of 17 genemonogenic diabetes panel. These results were further confirmed with Sanger sequencing.

Results: 9 out of 11 patients were homozygous for pathogenic/likely pathogenic variants in the *WFS1* gene. Interestingly, 3 of these probands were positive for the novel *WFS1* (NM_006005.3): c.1107_1108insA (p.Ala370Serfs*173) variant, and haplotype analysis suggested a founder effect in 3 families from Southern India. Additionally, we identified 2 patients with young-onset diabetes who were heterozygous for a likely pathogenic variant or a variant of uncertain significance in the *WFS1* gene.

Conclusion: These results project the need for NGS-based parallel multigene testing as a tool for early diagnosis of WFS and identify heterozygous *WFS1* variants implicated in young-onset diabetes.

Key Words: next-generation sequencing, Wolfram syndrome, founder mutation, DIDMOAD, *WFS1*

Abbreviations: NGS, next-generation sequencing; PCR, polymerase chain reaction; WFS, Wolfram syndrome; WS1, Wolfram syndrome type 1.

Wolfram syndrome type 1 (WS1) is a rare monogenic disorder also referred to as DIDMOAD (diabetes insipidus, diabetes mellitus, optic atrophy, and deafness) syndrome (1, 2). The underlying pathogenesis is ascribed to the mutations in the *WFS1* gene, which encodes a transmembrane protein Wolframin, primarily involved in membrane trafficking and endoplasmic reticulum homeostasis. This protein is highly expressed in brain tissues, pancreatic β-cells, and the heart. The *CISD2* gene is another gene implicated in Wolfram syndrome type 2, characterized by bleeding upper intestinal ulcers and defective platelet aggregation, but without diabetes insipidus and psychiatric disorders (3).

The first clinical manifestation in WS1 is nonautoimmune insulin–dependent diabetes; and, at a later stage, the onset of optic atrophy and sensorineural deafness (4, 5). However, the presentation of these clinical symptoms in patients with WS1 generally occurs below the age of 15 years (6, 7). Additionally, some of these patients may also present with conditions of endocrine dysfunction, and neurological and psychiatric abnormalities (8-11). WS1 is a debilitating disease, and an early diagnosis is crucial to enable appropriate prognostication, prevent complications, provide genetic counseling, and guide the family to reduce transmission to the future progeny (12). Recently the Food and Drug Administration has granted orphan drug designation for PB-TURSO, which is a combination of phenylbutyrate and tauroursodeoxycholic acid, for the treatment of Wolfram syndrome (WFS). With the availability of

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these alternative therapeutic options, an early confirmed genetic diagnosis may therefore potentially improve clinical management and may increase the rate of survival of these patients and pave a way for personalized therapy in the near future.

WS1 may be inherited in an autosomal recessive or dominant mode, and there are rare heterozygous variants reported in patients with diabetes (13, 14). Interestingly, the *WFS1* rare variant c.1672C>T (p.Arg558Cys) has been reported to be more common in the Ashkenazi Jewish population; additionally, the homozygous p.Arg558Cys variant was found to cause a milder form of WFS and the heterozygous carriers were found to be predisposed to diabetes (15). Therefore, there is a need for an unbiased approach for genetic screening in nonautoimmune forms of diabetes and young-onset diabetes to identify homozygous and heterozygous *WFS1* variants and their associated diabetes phenotypes.

Research Design and Methods

In this case series, we present 11 patients who were positive for *WFS1* variants, identified through targeted next-generation sequencing (NGS) of 17 genes implicated in syndromic forms of diabetes.

The inclusion criteria for the patients to be screened for targeted monogenic diabetes panel include

1. patients with a clinical suspicion of WFS (diabetes + deafness/optic atrophy)
2. Patients with nonautoimmune diabetes but with a strong family history
3. Young-onset diabetes patients who are negative for a 14-gene maturity onset diabetes of the young (MODY) panel.

Patient recruitment was carried out in the Department of Endocrinology, Christian Medical College, Vellore, and samples were also received on a referral mode from centers across India, Sri Lanka, and Nepal. Informed consent was obtained from all patients or parents/guardians, and the study had been approved by the Ethics Committee (IRB MIN NO/10813 dated 23/08/2017), Christian Medical College, Vellore.

Automated DNA extraction from the peripheral blood was carried out using the Promega Maxwell® RSC system (Promega Corporation, WI, USA). The quality and quantity were assessed using a Nanodrop 1000 spectrophotometer (Thermo Scientific, MA, USA) and Qubit fluorometer (Invitrogen, CA, USA). Multiplex polymerase chain reaction (PCR) coupled with an NGS-based workflow was utilized based on the published protocol (16). In short, the target enrichment was carried out using the in house–developed novel multiplex PCR-based enrichment for 17 genes (*AKT2*, *CISD2*, *CP*, *EIF2AK3*, *GATA6*, *GLUD1*, *HADH*, *IER3IP1*, *INSR*, *NEUROG3*, *PTF1A*, *RFX6*, *SLC2A2*, *WFS1*, *ZFP57*, *GLIS3*, *FOXP3*) using a QIAGEN® (Hilden, Germany) multiplex PCR kit, followed by sequencing on Ion Torrent™ PGM (Ion Torrent, Life Technologies, CA, USA).

Data analysis and coverage analysis were carried out on Ion Torrent suite software (Version 5.0.4.0) and DNA Star software (DNASTAR, WI, USA). Variant classification was based on ACMG (American College of Medical Genetics and Genomics) 2015 guidelines (17). The identified variants with pathogenic/likely pathogenic significance were further confirmed by Sanger sequencing.

Screening of Microsatellite Markers for the Probable Founder *WFS1* Mutation

The highly polymorphic microsatellite markers D4S2957, D4S2935, D4S3007, D4S394, D4S3023, D4S2925, D4S2285, and D4S431 flanking the *WFS1* gene mutation were selected from the UCSC Genome Browser, and primers were designed using the Primer3 site as per the published protocols (18, 19). Microsatellite markers were PCR amplified and were sequenced with Ion Torrent PGM using the Ion PGM™ 400 Sequencing Kit (Ion Torrent, Life Technologies, CA, USA), and microsatellite repeats were visually counted using the Integrative Genomics Viewer v2.9.4 (20).

Results

We have identified 11 patients with *WFS1* gene variants (Table 1); 6 of these patients were born to consanguineous parents (pedigrees Figs. 1 and 2), and the mean age of diagnosis of diabetes in patients with homozygous vs heterozygous variants was 6.7 and 27.5 years (Table 2). A total of 6 patients (5 homozygous and 1 heterozygous) were found to be positive for 4 novel variants. Patients with novel vs reported variants were diagnosed with diabetes mellitus at a mean age of 7.4 vs 6 years. Optic atrophy was diagnosed in all 4 (100%) subjects with reported variants at the mean age of 14.5 years whereas 4/5 (80%) subjects were identified with novel variants presented with optic atrophy at the mean age of 12.5 years. Among those with reported variants deafness was diagnosed in 2/4 (50%) subjects at the mean age of 18 years whereas only 1/5 (20%) subjects identified with novel variants presented with deafness diagnosed at the age of 8 years. None of the patients with reported variants were diagnosed with diabetes insipidus, whereas 2/5 (40%) subjects identified with homozygous novel variants presented with diabetes insipidus diagnosed at a mean age of 14.5 years.

Out of 11 patients, 3 were positive for a novel frameshift variant *WFS1*(NM_006005.3):c.1107_1108insA (p.Ala370SerfsTer173) and 2 were positive for a *WFS1*(NM_006005.3):c.1228_1231delCTCT (p.Val412SerfsTer29) variant. Out of 8 variants identified, 6 variants were found in exon 8, 1 variant each in exon 4, and exon 5.

Homozygous *WFS1* Variants

Probable founder *WFS1* mutation

Three subjects, W9, W10, W11, from unrelated families were found to be positive for a novel homozygous *WFS1*(NM_006005.3):c.1107_1108insA (p.Ala370SerfsTer173) frameshift mutation (Fig. 1), which results in a substitution of amino acid alanine to serine at codon 370 and also a premature termination at codon 543. On further evaluation, these patients were found to be from the same region and community. Additionally, we have investigated 12 members of these families. A total of 6 subjects (including 3 probands) were homozygous positive for the mutation, and all of them were diagnosed with diabetes at a mean age of 4.1 years; bilateral optic atrophy was diagnosed in all 6 members at a mean age of 11.6 years, and only 1 of the probands and his sibling were diagnosed with deafness at ages 8 and 5 years (Table 2). There were 8 heterozygous carriers in the family and 1 negative for the mutation, and all of them were normal. Based on the ACMG 2015 guidelines, this variant has been classified as pathogenic (Table 3).

Table 1. List of *WFS1* gene variants identified in the study

Subject	Exon	Nucleotide change	Amino acid change	Allele status	Type of mutation	Reported/Novel
W9	8	c.1107_1108insA	p.A370Sfs*173	Homo	Frameshift	Novel
W10	8	c.1107_1108insA	p.A370Sfs*173	Homo	Frameshift	Novel
W11	8	c.1107_1108insA	p.A370Sfs*173	Homo	Frameshift	Novel
W8	8	c.1228_1231delCTCT	p.V412Sfs*29	Homo	Frameshift	(José Luiz Pedroso et al., 2015) (21)
W17	8	c.1228_1231delCTCT	p.V412Sfs*29	Homo	Frameshift	(José Luiz Pedroso et al., 2015) (21)
W2	8	c.2642_2643delTC	p.F883Lfs*56	Homo	Frameshift	(Hansen et al., 2003) (22)
W7	4	c.387G>A	p.W129X	Homo	Nonsense	(Casey et al.,2004) (23)
W15	8	c.1961C>G	p.S654X	Homo	Nonsense	Novel
W1	8	c.2197T>G	p.C733G	Homo	Missense	Novel
W12	5	c.527_528insGCGC	p.K178Afs*70	Hetero	Frameshift	Novel
W3	8	c.1672C>T	p.R558C	Hetero	Missense	(Bansal et al., 2018) (15)

Figure 1. (A) Pedigrees of 3 families identified with founder mutation. (B) Sanger sequencing (highlighted in yellow) and (C) NGS Data showing homozygous insertion of Adenine nucleotide (highlighted in blue). (D) The genotypes of 8 microsatellite markers flanking *WFS1* mutation in 3 families suspicion of founder mutation. All the probands and their available family members share a common haplotype in the both the alleles for the microsatellite markers D4S2957, D4S2935, D4S3007, D4S394 (highlighted in brown) and 1 allele for the microsatellite markers D4S3023, D4S2925, D4S2285 and D4S431 (highlighted in blue). Except subject IIIC—W11 sibling who was negative for the mutation was a heterozygous carrier of 18 (CA) and 12 (TG) repeats for D4S2935 and D4S3007 (highlighted in green).

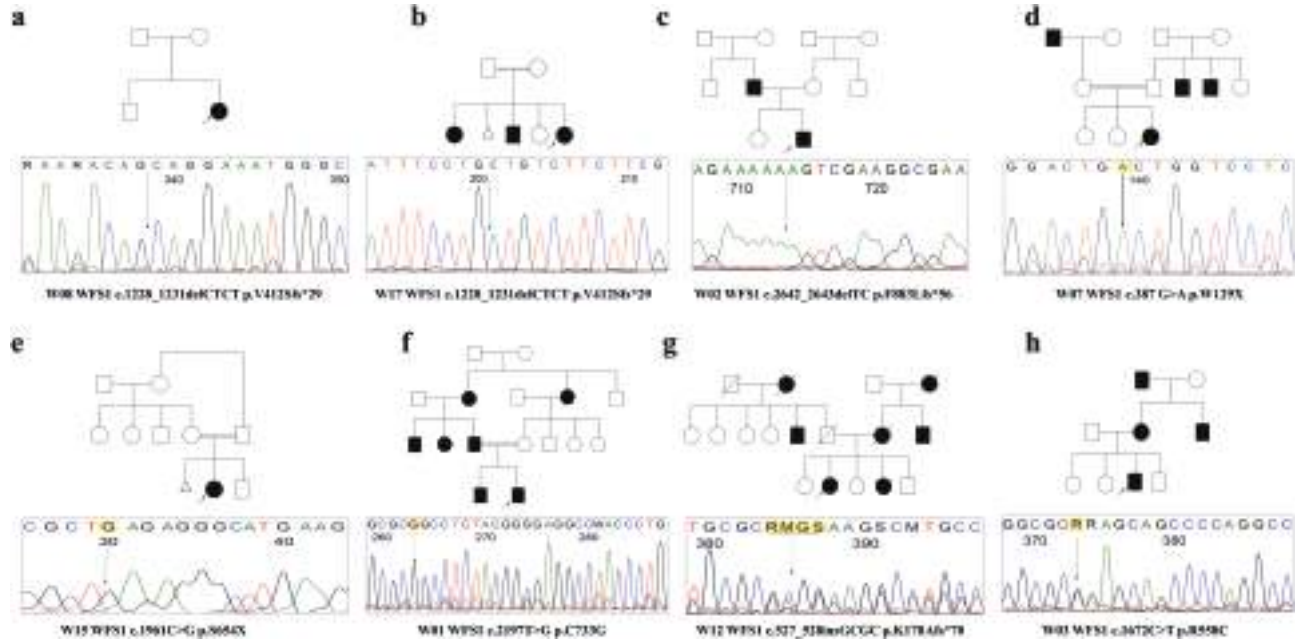

Figure 2. Pedigree(s) and Sanger data of the patients identified with *WFS1* variants. (A,B,C) Patients with homozygous frameshift mutation. (D,E) Patients with homozygous nonsense variant. (F) Patient with homozygous missense variant. (G) patient with heterozygous frameshift variant. (H) Patient with heterozygous rare missense variant. Substitutions and insertions are highlighted in yellow.

Eight microsatellite markers were screened to investigate the probable founder effect of the frameshift mutation identified in the study. Based on these investigations, the probands and their available family members were found to share a common haplotype in both the alleles for the microsatellite markers D4S2957, D4S2935 D4S3007, D4S394, and 1 allele for the microsatellite markers D4S3023, D4S2925, D4S2285, and D4S431. In addition, the subject (IIIC) who was negative for the mutation was a carrier of D4S2935 (18 CA repeats) and D4S3007 (12 TG repeat) markers (Fig. 1). These results suggest that the novel mutation identified in the study could be a founder mutation from Southern India.

Frameshift and nonsense mutations

W8 and W17 were homozygous for *WFS1* (NM_006005.3): c.1228_1231delCTCT (p.Val412SerfsTer29) variant. This is a reported mutation in *WFS1*-related spectrum disorders (24) and, in addition, these patients presented with pubertal delay. Based on the published literature and the ACMG guidelines, this variant has been classified as pathogenic (17).

Subject W2 was a 26-year-old male found to be positive for a homozygous *WFS1*(NM_006005.3): c.2642_2643del (p.Phe883LeufsTer56) variant resulting in a premature termination at codon 939. The proband was diagnosed with diabetes at the age of 7 years, with visual impairment at the age of 12 years and with minimal hearing loss at the age of 24 years.

Subject W7 was a 16-year-old female diagnosed with diabetes at the age of 7 years, and at the age of 12 she was diagnosed with deafness and psychiatric manifestations, including reactive depression and hypothyroidism, and was clinically suspected of having WFS. Surgery for bilateral cataracts was performed. On further genetic evaluation, she was positive for the *WFS1* gene–reported mutation *WFS1*(NM_006005.3):c.387G>A (p.Trp129Ter), which results in premature truncation of the protein. This variant has been previously reported in a patient with a severe form of WFS along with sleep apnea diagnosed at the age of 8 (23).

Subject W15 was a 13-year-old female diagnosed with diabetes at 13 and treated with insulin and presented with hypothyroidism, bilateral optic atrophy without deafness. The patient, was found to be positive for a novel homozygous *WFS1*(NM_006005.3): c.1961C>G (p.Ser654Ter) variant. The variant was inherited from her second-degree consanguineous parents, who were heterozygous carriers for the same mutation and were normal. However, the proband's mother had a history of spontaneous abortion and her younger sibling, whose mutation status is unknown, was reportedly normal.

Homozygous missense variants

Subject W1 is a 13-year-old male diagnosed as type 1 diabetes mellitus at 11, and his elder sibling was also diagnosed as type 1 diabetes mellitus at the age of 9 years. The proband and his sibling required insulin for glycemic control. These patients were born to consanguineous parents, and their father was found to have diabetes at the age of 45 years. With a family history of diabetes, the proband was initially tested for MODY-related variants and was negative for a 14-gene panel. However, with signs of a nonautoimmune form of diabetes, born to consanguineous parents, along with a diabetic sibling, the sample was further processed for screening the extended 17 gene panel covering the syndromic forms of diabetes. Through these investigations, the patient was found to be positive for a novel homozygous *WFS1* mutation *WFS1* (NM_006005.3): c.2197T>G (p.Cys733Gly) mutation. The proband's sibling was also homozygous positive for the mutation, and the parents were heterozygous carriers. Currently, the patient and his sibling have normal vision and hearing capabilities.

Table 2. Age of diagnosis and clinical characteristics of patients positive for *WFS1* variants

Subject	Variant identified	Family member	Gender	Age	Carrier status	ADM	Initial Diagnosis	CSW (age)	ADI	AOA	AHD	Current therapy	Other anomalies
W9	*WFS1*(NM_006005.3): c.1107_1108insA (p.Ala370SerfsTer173)	Proband	M	19	Homo	4	T1DM	Yes (19)	19	19	8	Insulin	Hydroureteronephrosis
		Father	M	44	Hetero	—	—	—	—	—	—	—	—
		Mother	F	38	Hetero	—	—	—	—	—	—	—	—
		Sibling 1	M	21	Hetero	—	—	—	—	—	—	—	—
		Sibling 2	M	17	Homo	4	T1DM	Yes (17)	—	12	—	Insulin	—
		Sibling 3	M	14	Homo	4	T1DM	Yes (14)	—	14	5	Insulin	Nystagmus from birth
W10	*WFS1*(NM_006005.3): c.1107_1108insA (p.Ala370SerfsTer173)	Proband	F	10	Homo	2	T1DM	Yes (10)	10	10	—	Insulin	Mild hydroureteronephrosis, early neurogenic bladder
		Father	M	38	Hetero	—	—	—	—	—	—	—	—
		Mother	F	28	Hetero	—	—	—	—	—	—	—	—
		Sibling 1	F	7	Homo	4	T1DM	Yes(7)	7	7	—	Insulin	—
		Sibling 2	F	3	Hetero	—	—	—	—	—	—	—	—
W11	*WFS1*(NM_006005.3): c.1107_1108insA (p.Ala370SerfsTer173)	Proband	M	22	Homo	7	T1DM	Yes (22)	—	8	—	Insulin	Seizures
		Father	F	59	Hetero	—	—	—	—	—	—	—	—
		Mother	M	51	Hetero	—	—	—	—	—	—	—	—
		Sibling 1	F	27	Negative	—	—	—	—	—	—	—	—
		Sibling 2	F	26	Expired	9	T1DM	—		8	20	Insulin	Seizures, pneumonia (died at age 26)
W8	*WFS1*(NM_006005.3): c.1228_1231delCTCT (p.Val412SerfsTer29)	Proband	F	16	Homo	6	T1DM	No	—	14	—	Insulin	Delayed puberty, nystagmus, and postsubcapsular cataract
W17	*WFS1* (NM_006005.3): c.1228_1231delCTCT (p.Val412SerfsTer29)	Proband	F	19	Homo	4	T1DM	Yes (19)	—	19	—	Insulin	Hypogonadism, delayed puberty, primary amenorrhea, absence of secondary sexual characteristics and OCD
W2	*WFS1*(NM_006005.3): c.2642_2643delTC (p.Phe883LeufsTer56)	Proband	M	26	Homo	7	T1DM	Yes (24)	—	12	24	Insulin	—
W7	*WFS1*(NM_006005.3): c.387G>A (p.Trp129Ter)	Proband	F	16	Homo	7	T1DM	Yes (16)	—	13	12	Insulin	Hydronephrosis, hypothyroidism, and bilateral cataract
		Father	M	UA	Hetero	—	—	—	—	—	—	—	—
		Mother	F	UA	Hetero	—	—	—	—	—	—	—	—
		Sibling	F	UA	Negative	—	—	—	—	—	—	—	—
W15	*WFS1*(NM_006005.3): c.1961C>G (p.Ser654Ter)	Proband	F	13	Homo	13	T1DM	Yes (13)	—	13	—	Insulin	Hypothyroidism
		Father	F	35	Hetero	—	—	—	—	—	—	—	—
		Mother	M	32	Hetero	—	—	—	—	—	—	—	—

Table 2. Continued

Subject	Variant identified	Family member	Gender	Age	Carrier status	ADM	Initial Diagnosis	CSW (age)	ADI	AOA	AHD	Current therapy	Other anomalies
W1	*WFS1*(NM_006005.3): c.2197T>G (p.Cys733Gly)	Proband	M	13	Homo	11	T1DM	No	—	—	—	Insulin	—
		Father	M	50	Hetero	45	T2DM	—	—	—	—	OAD	—
		Mother	F	47	Hetero	—	—	—	—	—	—	—	—
		Sibling 1	M	14	Homo	9	T1DM	—	—	—	—	Insulin	—
W12	*WFS1*(NM_006005.3): c.527_528insGCGC (p.Lys178AlafsTer70)	Proband	F	39	Hetero	39	T2DM/ MODY	N/A	—	—	—	OAD	Follicular adenoma
W3	*WFS1*(NM_006005.3): c.1672C>T (p.Arg558Cys)	Proband	M	17	Hetero	16	T2DM/ MODY	N/A	—	—	—	OAD	—
		Mother	M	UA	Hetero	35	T2DM/ MODY	N/A	—	—	—	OAD	—
		Sibling 1	M	13	Hetero	—	—	—	—	—	—	—	—
		Maternal grandfather	M	UA	Hetero	UA	T2DM/ MODY	N/A	—	—	—	UA	—
		Maternal grandmother	F	UA	Negative	—	—	—					
		Maternal uncle	M	UA	Hetero	UA	T2DM/ MODY	N/A	—	—	—	UA	—

Abbreviations: ADM, age of diagnosis of diabetes mellitus; ADI, age of diagnosis of diabetes insipidus; AOA, age of diagnosis of optic atrophy; AHD, age of diagnosis of hearing defect; CSW (age), age at which clinical suspicion of Wolfram syndrome was made; MODY, maturity-onset diabetes of the young; OAD, oral antidiabetic drugs; OCD, obsessive compulsive disorder; T2DM, type 2 diabetes mellitus; UA, unavailable; N/A, not applicable.

Table 3. Interpretation and classification of *WFS1* variants based on ACMG 2015 guidelines

No.	Variant	Zygosity	Clinical diagnosis	Evidences of pathogenicity						Combining criteria	ACMG classification
1	p.A370Sfs*173	Homozygous	DIDMOAD	PVS1	PM2	PM3	PP1	PP4		1 PVS1 and ≥2 PM	Pathogenic
2	p.V412Sfs*29	Homozygous	DIDMOAD	PVS1	PM2	PM3	PP1	PP4	PP5	1 PVS1 and ≥2 PM	Pathogenic
3	p.F883Lfs*56	Homozygous	Type 1 diabetes mellitus	PVS1	PM3	PP1	PP4	PP5		1 PVS1 and ≥2 PM	Pathogenic
4	p.W129*	Homozygous	DIDMOAD	PVS1	PM2	PM3	PP1	PP4	PP5	1 PVS1 and ≥2 PM	Pathogenic
5	p.S654*	Homozygous	DIDMOAD	PVS1	PM2	PM3	PP1	PP4		1 PVS1 and ≥2 PM	Likely pathogenic
6	p.C733G	Homozygous	Type 1 diabetes mellitus	PM1	PM2	PM3	PP1	PP2	PP3 PP4	≥3 PM	Likely pathogenic
7	p.K178Afs*70	Heterozygous	Young-onset DM	PVS1	PM2					1 PVS1 and ≥2 PM	VUS[a]
8	p.R558C	Heterozygous	Young-onset DM	PS4	PP1	PP2	PP3	PP4	PP5	1 PS and ≥2 PP	Likely pathogenic

PM, moderate evidence of pathogenicity; PM1, variant located in mutational hotspot; PM2, identified variant is absent or extremely rare (<0.004%) from large population studies; PM3, detected in trans with a pathogenic variant for recessive disorders; PP, supporting evidence of pathogenicity; PP1, cosegregation with disease in multiple affected family members; PP2, low rate of benign missense variants in *WFS1* gene; PP3, multiple lines of computational evidence support a deleterious effect on the gene or gene product; PP4, patient phenotype or family history is highly specific for a disease with single genetic etiology; PP5, reported as pathogenic by reputed sources; PS, strong evidence of pathogenicity; PS4, prevalence of the variant is increased in affected individuals compared to controls; PVS, very strong evidence of pathogenicity; PVS1, null variant in gene with established loss function as disease mechanism.
Abbreviations: DIDMOAD, diabetes insipidus, diabetes mellitus, optic atrophy, and deafness; DM, diabetes mellitus.
[a]Heterozygous Variant, hence classified as variant of uncertain significance (VUS).

Heterozygous *WFS1* Variants in Young-onset Diabetes

The following patients were also tested for MODY genes and were negative for the 14 MODY gene panel. However, with a strong family history, the samples were further processed for the 17 gene panel.

Subject W12 is from Sri Lanka with a body mass index of 21 kg/m^2, and was diagnosed with diabetes at 39 years of age. Although there was a 3-generation family history, mother with diabetes, sibling (diagnosed at the age of 35 and 27 years), and a maternal uncle with diabetes, she was negative for MODY-related mutations.

However, when screened for an extended monogenic diabetes 17 gene panel, she was found to be heterozygous positive for a novel *WFS1* frameshift mutation *WFS1* (NM_006005.3):c.527_528insGCGC (p.Lys178AlafsTer70), resulting in the substitution of lysine with arginine at codon 178 and a frameshift resulting in premature termination at codon 248. She is being treated with metformin and gliclazide with optimal glycemic control. Since the patient is negative for 30 monogenic diabetes genes and reports of *WFS1* heterozygous variants reported in diabetes, variants such as these need to be screened in family members and require further evaluation.

Subject W3 is a 17-year-old male from Nepal with a body mass index of 30.5 kg/m^2 who tested positive for a heterozygous *WFS1*(NM_006005.3):c.1672C>T (p.Arg558Cys) mutation. He was diagnosed with diabetes at the age of 16 and treated with metformin and glimepiride to achieve glycemic control. The subject's mother was heterozygous positive for the same mutation and was diagnosed with diabetes at 35 years of age. On further family screening, the proband's maternal grandfather and a maternal uncle who had diabetes were heterozygous carriers for the variant, the maternal nondiabetic grandmother was negative for the variant. However, the proband's younger sibling who is 13 years old is also a heterozygous carrier, but with normoglycemia at this point in time. The other 2 siblings were not available for genetic screening.

Discussion

Rare monogenic forms of diabetes, like WFS with progressive neurological abnormalities, pose a significant challenge in early diagnosis and management (25). In this study, 3 individuals from unrelated families were positive for a novel *WFS1* homozygous mutation *WFS1*(NM_006005.3):c.1107_1108insA (p.Ala370SerfsTer173). Based on the data from screening 8 microsatellites, we report this mutation as a *WFS1* founder mutation from southern India. To the best of our knowledge, this is the first report of founder mutation identified in patients with WFS. With a high rate of consanguineous and closed community marriages, there could be an increased number of individuals carrying risk alleles for various rare recessive disorders like WFS in India (26).

We have also identified a *WFS1* recurrent mutation *WFS1*(NM_006005.3): c.1228_1231delCTCT (p.Val412SerfsTer29) in 2 patients from the neighboring states of Southern India (on the Tamil Nadu-Andhra Pradesh border) associated with a DIDMOAD phenotype along with hypogonadism and pubertal delay, which may be associated with WFS (24). This variant has been reported earlier in 8 individuals from unrelated families in Brazil, and there

is further evidence from the functional data confirming its pathogenicity in WFS (27, 28).

Subject W1 tested negative for the MODY gene panel; however, with a sibling with diabetes and born to consanguineous parents, the patient was further evaluated for an extended panel of genes implicated in syndromic forms of diabetes and was found to be positive for a novel nonsynonymous homozygous variant *WFS1*(NM_006005.3):c.2197T>G (p.Cys733Gly). Homozygous missense variants are also known to cause milder forms of WFS (15) and the severity was based on the location of the mutation (29). The patient with variants in the luminal domain have been reported with an early-onset classical phenotype, and those in the transmembrane and cytosolic domain have been reported to present with milder phenotypes and late-onset of symptoms (30). *WFS1*(NM_006005.3): c.2197T>G (p.Cys733Gly) mutation is in the luminal domain and is expected to cause a severe form of the disease. Therefore an early diagnosis of WS1 may delineate a way for personalized therapy in the near future (31).

In this study, a young patient with diabetes who was positive for *WFS1* rare missense variant *WFS1*(NM_006005.3):c.1672C>T (p.Arg558Cys) was initially diagnosed as type 2 diabetes with a body mass index of 30.5 kg/m^2 and without optic atrophy. Interestingly this variant has also been found to be more common (60-fold higher) in subjects with an Ashkenazi Jewish ancestry with Minor Allele Frequency: 0.14, and was shown to cause a milder clinical phenotype of WFS. Furthermore, these patients were diagnosed with young-onset diabetes (17.8 ± 8.3 years); however, with a reduced penetrance for optic atrophy. Additionally, the case–control investigations among Ashkenazi Jewish ancestry demonstrated that heterozygous carriers were at an increased risk for type 2 diabetes (OR 1.81, P = .004) (15).

Heterozygous variants have also been reported to cause nonsyndromic sensorineural hearing loss (32) and autosomal dominant optic atrophy and hearing loss (33). De novo heterozygous missense variants are also reported in patients who were diagnosed with diabetes before 12 months, sensorineural deafness soon after birth, congenital cataract, and hypotonia. Unusual clinical presentations of WFS were reported in families with autosomal forms of congenital nuclear cataracts and autosomal dominant young-onset diabetes (34). However, in this study the patients with heterozygous variants presented with only young-onset diabetes with a strong family history but without any other complications. Interestingly, the patient with the *WFS1* heterozygous Arg558Cys variant had a very high body mass index.

These findings, we believe have major significance, since even the low penetrant heterozygous MODY gene variants have been reported in patients who might require additional environmental or have genetic factors that cause young-onset diabetes (16, 35, 36). The highest prevalence of *WFS1* variants in certain populations may be due to occurrence of high rate of consanguineous marriages (37-40). Therefore, rare variants enriched in a population that are functionally important in beta cell development or functioning may be misclassified or filtered off even with evidence of pathogenicity from functional assays. Therefore, there is a need for extensive population studies to identify rare variants with pathogenic significance in young-onset diabetes in India and other developing countries (41).

Conclusion

In this study, parallel multigene testing has shown to be a robust tool for early diagnosis of syndromic and nonsyndromic forms of diabetes due to *WFS1* mutations. Through these investigations, we have identified 3 families with a novel founder mutation from Southern India. Moreover, we have identified a novel heterozygous *WFS1* variant in patient with young-onset diabetes which corroborates the importance of utilizing comprehensive genetic screening panels in precision medicine.

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Disclosure Summary

The Authors declare that there is no conflict of interest associated with this publication.

Data Availability

We have submitted the NGS raw data to the European Nucleotide Archive and the details are given below. Study accession number is: PRJEB48305. Study unique name is: ena-STUDY-CHRISITIAN MEDICAL COLLEGE-25-10-2021-11:26:30:344-502. Sample accession Secondary Accession Sample unique name ERS8120195 SAMEA10468024 Sample_WFS1 ERS8120197 SAMEA10468026 Sample_WFS7 ERS8120198 SAMEA10468027 Sample_WFS8 ERS8120199 SAMEA10468028 Sample_WFS9 ERS8120196 SAMEA10468025 Sample_WFS12 ERS8120200 SAMEA10468029 Sample_WFS15 ERS8120201 SAMEA10468030 Sample_WFS17 ERS8120202 SAMEA10468031 WFS9_Haplotype ERS8120203 SAMEA10468032 WFS10_Haplotype ERS8120204 SAMEA10468033 WFS11_Haplotype. All other relevant data are included within the paper.

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